J. Physiol. (1982), **331**, *pp.* 547–555
With 5 text-figures
Printed in Great Britain

ROLE OF CYCLIC ADENOSINE MONOPHOSPHATE IN AMYLASE RELEASE FROM DISSOCIATED RAT PANCREATIC ACINI

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(*Received 8 September 1981*)

SUMMARY

1. The effect of octapeptide of cholecystokinin-pancreozymin (CCK_8), bethanechol, cholera toxin, glucagon and vasoactive intestinal polypeptide (VIP) on amylase secretion and lactic dehydrogenase (LDH) release from isolated rat pancreatic acini was studied.

2. In isolated rat pancreatic acini, in the absence of theophylline in the medium, amylase secretion was increased by 65–78% with 10^{-7} and 10^{-6} M-cholera toxin. In the presence of theophylline, amylase secretion was increased by 43–56% with 10^{-7} and 10^{-6} M-cholera toxin following a 90 min incubation. No effect was observed in the presence of theophylline at 30 and 60 min. The effect of cholera toxin was potentiated by CCK_8 at 60 and 90 min.

3. In the absence of theophylline in the medium, amylase secretion was increased by 81–118% with 10^{-5} and 10^{-4} M-glucagon and 86% with 10^{-6} M-VIP at 60 min. In the presence of theophylline in the medium, amylase secretion was increased by 53–246% with 10^{-9} to 10^{-6} M-glucagon and 111–158% with 10^{-7} and 10^{-6} M-VIP respectively. The effect of glucagon and VIP was potentiated by CCK_8.

4. Potentiation of the rate of amylase release due to glucagon (10^{-5} M) and VIP (10^{-6} M) occurred during the first 15 min of incubation.

5. Release of LDH was not increased by any of these agents.

6. It is concluded that cyclic AMP rise (due to cholera toxin, glucagon and VIP effect) increased amylase secretion from rat pancreatic acinar cells. This effect is less marked than in the guinea-pig pancreas and is potentiated by agents mobilizing cellular Ca^{2+} (CCK_8 and bethanechol).

7. These data indicate species-specific variation in the action of cyclic AMP in the pancreas.

INTRODUCTION

In the guinea-pig pancreas, secretin, vasoactive intestinal polypeptide (VIP) and cholera toxin were reported to increase cellular cyclic AMP and stimulate enzyme secretion (Gardner, Conlon & Adams, 1976; Robberecht, Conlon & Gardner, 1976; Gardner & Jackson, 1977; Gardner & Rottman, 1979). Gardner & Jackson (1977) postulated that cyclic AMP (elaborated by acinar cells or contaminating duct cells) was involved in one of the major pathways for the regulation of digestive enzyme secretion from pancreatic acinar cells. In the rat pancreas, cholera toxin-induced increase in cyclic AMP was not accompanied by an increase in pancreatic enzyme secretion (Kempen, de Pont & Bonting, 1975; Smith & Case, 1975; Singh, 1979), whereas secretin, porcine VIP and exogenous derivatives of cyclic AMP increased pancreatic enzyme secretion (Robberecht, Deschodt-Lanckman, De Neef & Christophe, 1974; Heisler, Grondin & Forget, 1974; Deschodt-Lanckman, Robberecht, De Neef, Labrie & Christophe, 1975; Kempen, de Pont & Bonting, 1977; Bauduin, Stock, Launay, Vincent, Potvliege & Grenier, 1977). The lack of enzyme secretion was noted in spite of cholera toxin molecule being active biologically. Robberecht, Deschodt-Lanckman, Lammens, De Neef & Christophe (1977) reported that rat, mouse, guinea-pig, cat and dog pancreas responded to secretin and VIP with increased levels of cyclic AMP but enzyme secretion was stimulated only in the rat and guinea-pig pancreas. These findings could explain the lack of an effect of cholera toxin on enzyme secretion from cat pancreas (Smith & Case, 1975), but not from rat pancreas (Smith & Case, 1975; Kempen *et al.* 1975; Singh, 1979). It is of interest that most of the studies implicating cyclic AMP in enzyme secretion in guinea-pig pancreas used dissociated cells or acini, whereas studies implicating cyclic AMP in secretion of amylase from rat pancreas were done *in vivo* or *in vitro* slices. Dissociated acini were reported to be more responsive to secretagogues than single acinar cells and tissue slices in guinea-pig and rat pancreas (Robberecht *et al.* 1977; Gardner & Jackson, 1977; Peikin, Rottman, Batzri & Gardner, 1978; Williams, Korc & Dormer, 1978). Therefore the action of peptides reported to increase cyclic AMP was studied to define the role of cyclic AMP in the digestive enzyme secretion from rat pancreatic acinar cells. In addition, potentiating interaction between second messengers involved in enzyme secretion reported previously in dissociated acinar cells from guinea-pig pancreas by Gardner & Jackson (1977) and by the author in rat pancreatic lobules (Singh, 1979) was studied.

METHODS

The following were purchased: Male Sprague Dawley rats from ARS/Sprague Dawley and Holtzman Co., Madison, WI, U.S.A.; chromatographically purified collagenase from Millipore, Freehold, NJ, U.S.A.; α-Chymotrypsin from Worthington Biochemical Corp., Freehold, NJ, U.S.A.; hyaluronidase, bovine plasma albumin (fraction V), soybean trypsin inhibitor, L-glutamine, β-nicotinamide adenine dinucleotide (DPN), cholera toxin (vibrio cholera, lyophilized powder prepared and purified according to Mekalanos, Collier & Romig (1978) from Sigma Chemical Co., St. Louis, MO, U.S.A.; essential amino acids (Eagle-100 X concentrated) from Grand Island Biological Co., Grand Island, NY, U.S.A.; glucagon from Eli Lilly Co., Norcross, GA, U.S.A.; cholecystokinin (fragment 26–33) octapeptide, vasoactive intestinal polypeptide (VIP) from Boehringer Mannheim Biochemical Co., Indianapolis, IN, U.S.A.; bethanechol chloride (BC) from Merck, Sharp and Dohme Co., West Point, PA, U.S.A. All other chemicals were reagent grade.

Preparation of dissociated acini

In preliminary experiments acinar cells and lobules were isolated from male Sprague Dawley rats (average weight 200 g, fasted overnight) by methods used previously (Singh, 1979, 1980*a*, *b*). For dissociation of acini, animals were killed by a blow to the head and exsanguinated. The pancreas was removed, trimmed free of mesentery, fat and lymph glands, and weighed (1–1·2 g wet weight). The composition of standard incubation medium for dissociation of acini was (mM): NaCl, 118; KCl, 4·7; $MgCl_2$, 1·2; $NaHCO_3$, 25; KH_2PO_4, 1; glucose, 14; glutamine, 2; with 0·01 % (w/v) chromatographically purified soybean trypsin inhibitor and 2 % (v/v) essential amino acid mixture (Eagle, 1959). The glands were injected with 5 ml. enzyme solution (standard incubation medium containing 0·1 mM-$CaCl_2$ with 45 units $ml.^{-1}$ pure collagenase, 0·04 mg $ml.^{-1}$ trypsin, and 1·8 mg $ml.^{-1}$ hyaluronidase). Methodology for incubation, purification and establishing functional capacity of acinar preparation was similar to that described by Williams *et al.* (1978). The final preparation was suspended in 1:10 dilution in standard containing 1·25 mM-$CaCl_2$ and 1 % albumin (KHB medium).

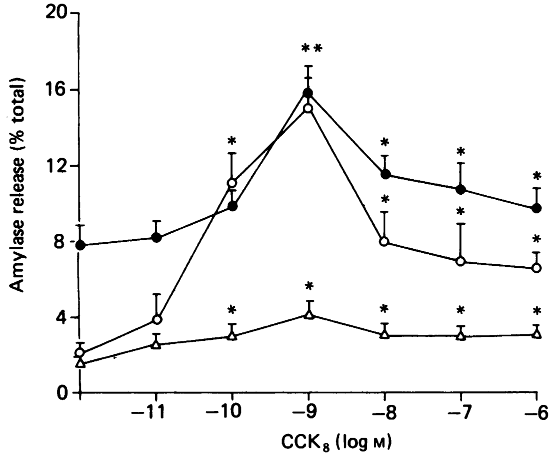

Fig. 1. Concentration dependence of amylase release stimulated by CCK_8 (10^{-11}–10^{-6} M) from dissociated acini, acinar cells and lobules (○——○, △——△, ●——●, respectively) from rat pancreas following 60 min incubation. Amylase release in media was expressed as percent of total amylase in the samples. All values are mean ± S.E. of mean of four to twelve experiments (*significantly different from controls).

Amylase and LDH release

After pre-incubation for 60 min, the samples of acinar suspension were poured into graduated centrifuge tubes and spun for 2 min at 100 *g* and resuspended in KHB medium in 1:10 dilution. After a thorough mixing of the acini with the solution, ½ ml. aliquots of suspension of dissociated acini were distributed in incubation flasks, secretagogues added, and the final incubation volume made up to 5 ml. with KHB medium. An aliquot of the suspension was centrifuged and the medium saved to determine the amylase and LDH at the start of incubation. Following incubation with secretagogues an aliquot from each flask was centrifuged in Eppendorf tubes in a Beckman microfuge at 11,000 *g* for 1 min and both the pellets and the medium were saved for amylase and LDH assays. Amylase content of the media and sonicated acini were determined by the method described by Bernfeld using Lintner's starch as substrate (Bernfeld, 1955). The initial values of amylase were subtracted from the values obtained following incubation with secretagogues to determine the release of amylase during the period of stimulation. One unit of amylase is the amount of starch hydrolysed to 1 mg maltose in 3 min at 37 °C. Lactic dehydrogenase was assayed spectrophotometrically by the methods used previously (Singh, 1979). Amylase and LDH in the media were expressed as percent of total in the samples.

Statistical analysis

Data were analysed by Student's *t* test for paired values (Snedcor & Cochran, 1975).

RESULTS

Preliminary experiments revealed that dispersed acini from rat pancreas were much more responsive than dispersed single cells. During a 60 min incubation, basal secretion of amylase from dispersed cells amounted to 1·6 ± 0·2 % (± S.E.M.) and there

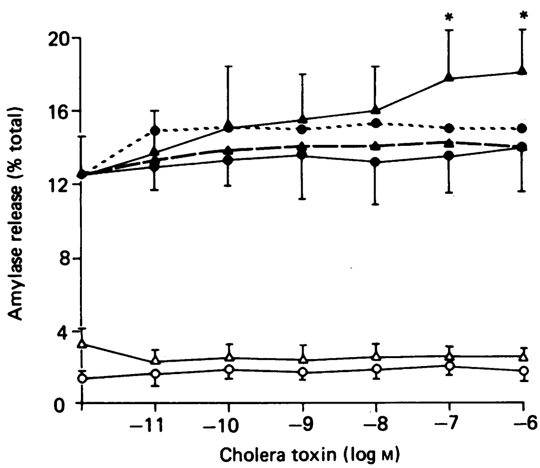

Fig. 2. Concentration dependence of amylase release stimulated by cholera toxin alone or with CCK_8 (10^{-9} M) with a 30 min incubation (○——○, ●——●, respectively) or a 60 min incubation (△——△, ▲——▲, respectively) in the presence of theophylline (5 mM) in the medium. Dashed lines indicate the sums of effects of CCK_8 (10^{-9} M) with various concentrations of cholera toxin separately. All values are mean ± S.E. of mean of five experiments (*significantly different from controls).

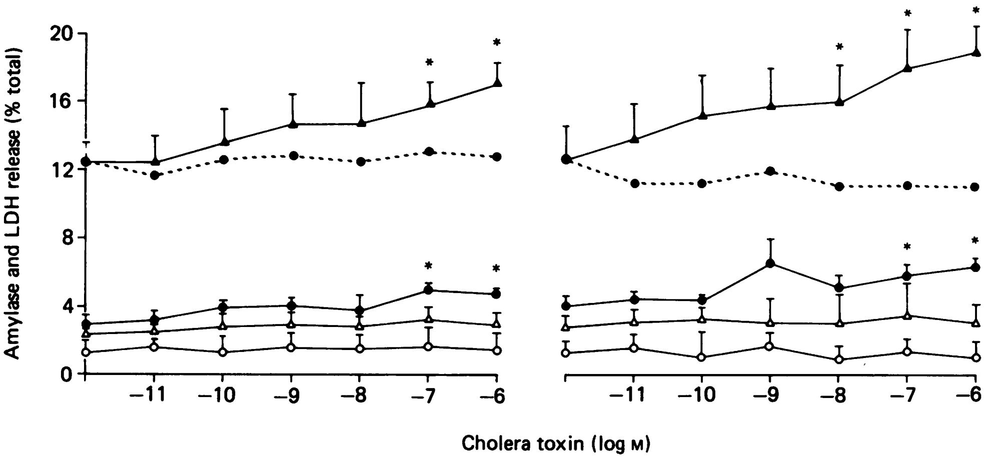

Fig. 3. Concentration dependence of amylase and LDH release stimulated by cholera toxin alone (●——●, ○——○, respectively) or with 10^{-9} M-CCK_8 (▲——▲, △——△, respectively) in the absence of theophylline in the medium (left panel) and in the presence of theophylline (5 mM) in the medium (right panel) following a 90 min incubation. Dashed lines shown in both panels indicate the sums of the effect of CCK_8 (10^{-9} M) with various concentrations of cholera toxin separately. All values are mean ± S.E. of mean of five experiments (*significantly different from controls).

was a 74 % increase with CCK_8 (10^{-9} M). With dissociated acini, the basal secretion was 2·1 ± 0·4 % and there was a 615 % increase with CCK_8 (10^{-9} M). With dissociated lobules, basal secretion was 7·8 ± 0·9 % and there was a 100 % increase with CCK_8 (10^{-9} M). All three preparations were comparable as far as dose–response curves and maximally effective concentrations were concerned (see Fig. 1).

At 30 and 60 min, cholera toxin, in the presence of 5 m-mole of theophylline in the medium, did not increase amylase secretion (See Fig. 2). At 60 min (but not at 30 min) there was potentiation of the effect of CCK_8 (10^{-9} M) with 10^{-7} and 10^{-6} M-cholera toxin. Although not shown in the Figure, release of LDH was not increased by these agents alone or in combination.

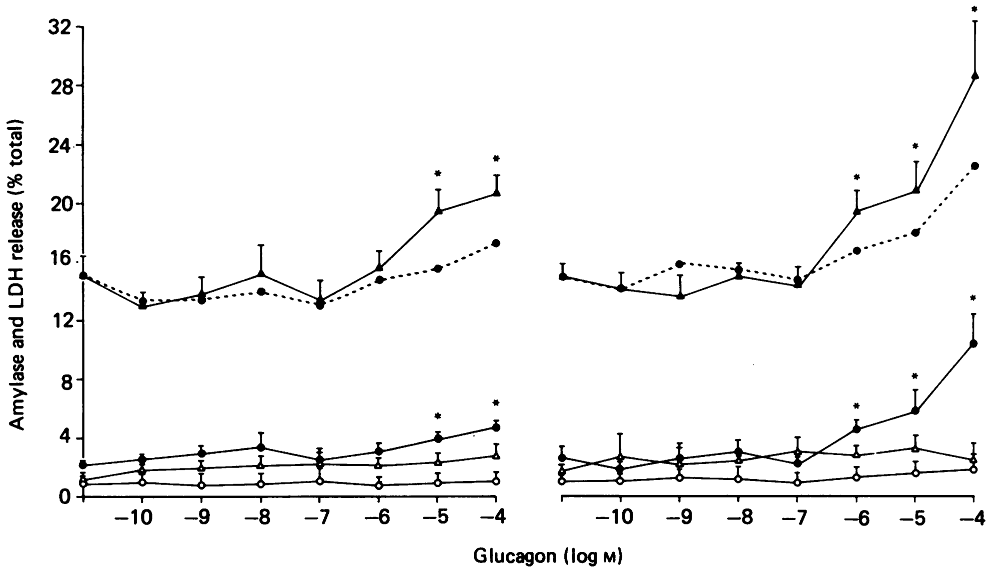

Fig. 4. Concentration dependence of amylase and LDH release stimulated by glucagon alone (●——●, ○——○, respectively) or with 10^{-9} M-CCK_8 (▲——▲, △——△, respectively) in the absence (left panel) and in the presence of theophylline (5 mM) in the medium (right panel) following a 60 min incubation. Dashed lines shown in both panels indicate the sums of the effect of CCK_8 (10^{-9} M) with various concentrations of glucagon. All values are mean ± S.E. of mean of five to sixteen experiments (*significantly different from controls).

At 90 min and in the absence of theophylline in the medium, cholera toxin increased amylase secretion significantly at concentrations of 10^{-7} and 10^{-6} M (65–78 %). CCK_8 (10^{-9} M) potentiated the effect of cholera toxin at concentrations of 10^{-7} and 10^{-6} M, (left panel, Fig. 3). In the presence of theophylline in the medium, cholera toxin increased amylase secretion significantly in concentrations from 10^{-7} to 10^{-6} M (43–56 %). CCK_8 (10^{-9} M) potentiated the effect of cholera toxin at concentrations of 10^{-8} to 10^{-6} M (right panel, Fig. 3). The release of LDH was not significantly increased by cholera toxin alone or in combination with CCK_8.

Glucagon increased amylase secretion significantly at concentrations of 10^{-5} and 10^{-4} M (81–118 %) (See Fig. 4) in the absence of theophylline in the medium. Effect of glucagon at concentrations of 10^{-5} and 10^{-4} M was potentiated by CCK_8 (10^{-9} M) (left panel, Fig. 4). Glucagon increased amylase secretion significantly in concentrations from 10^{-6} to 10^{-4} M (53–246 %) in the presence of theophylline in the medium. Effect of glucagon at concentrations of 10^{-6} to 10^{-4} M was potentiated by CCK_8 (10^{-9} M) (right panel, Fig. 4). The release of LDH was not significantly increased by glucagon alone or in combination with CCK_8.

Vasoactive intestinal polypeptide increased amylase secretion significantly at a concentration of 10^{-6} M (86%) (see Fig. 5) in the absence of theophylline in the medium. Combination of CCK_8 (10^{-9} M) with various concentrations of VIP resulted in potentiation starting at 10^{-9} M, with significant potentiation observed at 10^{-7} and 10^{-6} M (left panel, Fig. 5). VIP increased amylase secretion significantly in concentrations from 10^{-7} to 10^{-6} M (111 and 156%, respectively) in the presence of

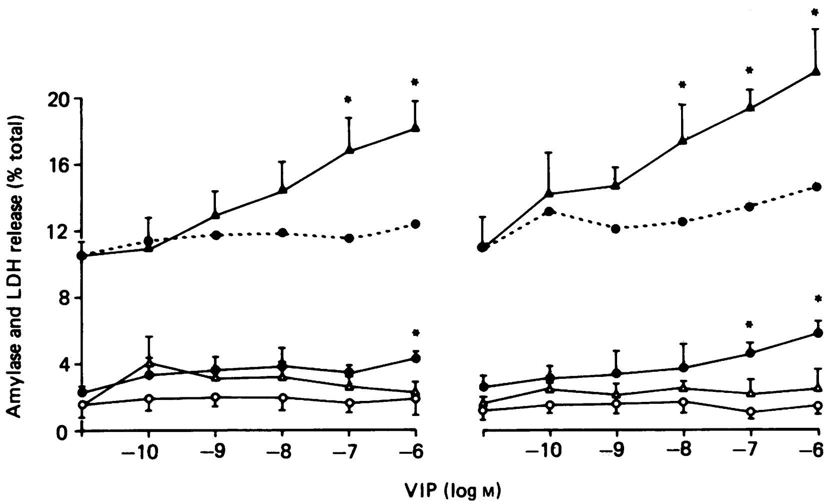

Fig. 5. Concentration dependence of amylase and LDH release stimulated by VIP alone (●——●, ○——○, respectively) or with 10^{-9} M-CCK_8 (▲——▲, △——△, respectively) in the absence (left panel) and in the presence of theophylline (5 mM) in the medium (right panel) following a 60 min incubation. Dashed lines shown in both panels indicate the sums of the effect of CCK_8 (10^{-9} M) with various concentrations of VIP separately. All values are mean ± S.E. of mean of five to six experiments (*significantly different from controls).

theophylline in the medium. With CCK_8, potentiation of the effect of VIP started at 10^{-10} M, but was significant at 10^{-8} to 10^{-6} M concentrations of VIP (right panel, Fig. 5). LDH release was not significantly increased with VIP alone or in combination with CCK_8.

Results of studies designed to examine the time course of secretion with CCK_8, glucagon and VIP, and of potentiation of effects of glucagon and VIP with CCK_8 are shown in Table 1. Secretion of amylase was determined individually for time periods of 15 min each at 15, 30, 45 and 60 min of incubation. CCK_8 increased secretion during each of the 15 min time periods for the duration of the incubation, glucagon for the first two time periods and VIP for the first 15 min only. However, potentiation of the effect of both of these agents was seen during the first 15 min only.

DISCUSSION

It was postulated that pancreatic digestive enzyme secretion is controlled by two pathways: one mediated by mobilization of intracellular Ca^{2+} activated by cholecystokinin and cholinergic agents, and the second mediated by a rise in cellular cyclic AMP activated by secretin and related peptides (Gardner & Jackson, 1977). Whereas the role of Ca^{2+} in amylase secretion is well established, the role of cyclic AMP in digestive enzyme secretion has been questioned (See Case, 1978). Robberecht *et al.* (1974) and Deschodt-Lanckman *et al.* (1975) reported that secretin and VIP increased cellular cyclic AMP levels and digestive enzyme content in secretion from cannulated pancreatobiliary ducts of rats and from rat pancreas fragments. Due to the type of models employed, these results could be explained on the basis of wash-out

TABLE 1. Effect of CCK_8, Glucagon and VIP on the time course of amylase release from isolated acini from rat pancreas

Experimental condition (*n*)	Time (min) 15	30	45	60
Control (13)	0·73±0·22	1·53±0·30	1·43±0·52	0·82±0·40
CCK_8 (10)	5·45±0·45****	6·75±0·80	4·81±0·72****	3·68±1·16*
Glucagon (5)	1·90±0·39**	3·88±0·74**	1·76±0·82	1·36±0·51
VIP (5)	1·78±0·27***	1·42±0·36	1·80±1·02	1·44±0·46
Glucagon + CCK_8 (8)	10·21±0·62†	7·06±1·41	6·16±2·33	3·46±3·09
VIP + CCK_8 (5)	8·40±0·63†	5·58±0·83	5·62±0·54	5·94±1·27

Results shown are mean ± S.E.M. of five to thirteen experiments. Data are expressed as amylase secretion as percent of total in the samples.

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·005$; **** $P < 0·001$.

† Potentiation of glucagon and VIP effect with CCK_8.

of digestive enzymes from ducts by increased electrolyte secretion. In addition, reported effects of dibutyryl cyclic AMP (Morisset & Webster, 1971; Bauduin *et al.* 1977) and phosphodiesterase inhibitors (Heisler *et al.* 1974) on amylase release as evidence for the role of cyclic AMP in enzyme secretion were questionable because of cell damage as indicated by excessive LDH release from acinar cells (Kempen *et al.* 1977). In a previous publication and in agreement with others (Singh, 1979; Smith & Case, 1975; Kempen *et al.* 1975), it was reported that cholera toxin increased cellular cyclic AMP in the rat pancreatic fragments, but the rise in cyclic AMP did not result in increased amylase secretion. However, potentiation of amylase secretion due to the ionophore A23187 with cholera toxin was reported (Singh, 1979). It was suggested that these findings argued against an important role for cyclic AMP by itself in the stimulation of pancreatic enzyme secretion (Singh, 1979). Later, this author reported that glucagon increased amylase secretion from dissociated mouse pancreatic acinar cells and acini (Singh, 1980*a*). It was shown that the action of glucagon was not mediated by a rise in cellular Ca^{2+} due to mobilization of intracellular stores or influx of extracellular Ca^{2+} (Singh, 1980*c*). It is now known that glucagon (like VIP and secretin) stimulates pancreatic enzyme secretion in dissociated acini from guinea pig pancreas due to an increase in cellular cyclic AMP (Pandol, Jensen & Gardner, 1981).

This author's previous studies on cholera toxin were done on pancreatic lobules which is a much less responsive model than dissociated acini (Fig. 1). Since no reports of the effect of glucagon and VIP on dissociated rat pancreatic acini are available, the present study was done and shows that cholera toxin as well as glucagon and VIP increased enzyme secretion from the dissociated rat pancreatic acini and potentiated the effect of CCK_8. Addition of CCK_8 to these agents shifted the dose–response curve upward. These results were not due to the cell damage by the secretagogues since LDH release was not increased. In the presence of theophylline in the medium, cholera toxin, glucagon and VIP alone or in combination with CCK_8 elicited the same response as in the absence of the theophylline but at a lower dose. The theophylline-induced shift of the dose–response curve is consistent with previous reports of high concentrations of xanthine-inhibitable cyclic nucleotide phosphodiesterase activity in the rat pancreas (Rutten, Schoot, De Pont & Bonting, 1973). In contrast to guinea-pig pancreas, which responds with a 6–7-fold increase in amylase secretion to VIP and secretin, respectively, rat pancreas is much less responsive to the agents increasing cellular cyclic AMP both in magnitude and duration of response (see Table 1). An agent that mobilizes cellular calcium is required to amplify the action of cyclic AMP. However, the kinetics of potentiation of the glucagon and VIP effect with CCK_8 were similar to the kinetics of potentiation of VIP effect with CCK_8 in the guinea-pig pancreas (Peikin *et al.* 1978). This species-specific variation in cyclic AMP action was also alluded to recently by Bommelaer, Rozental, Bernier, Vaysse & Ribet (1981) in isolated dog pancreatic acini because secretin and VIP failed to stimulate amylase release from the dog pancreatic acini isolated by these authors according to the technique described by Amsterdam, Solomon & Jamieson (1978).

The author wishes to express his appreciation to Ms Anne Marie Fair for secretarial assistance and Ms Marilyn M. LaSure for technical assistance. This investigation was made possible by the Medical Research Service of the Veterans Administration.

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